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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
STANDLEY, STEVEN H	
ART UNIT	**PAPER NUMBER**
1649	

DATE MAILED: 11/17/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/705,985	ANDERSON ET AL.	
	Examiner	Art Unit	
	Steven H. Standley	1649	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *8/5/05*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-5,9,11,13,15-18,20,21,25 and 26* is/are pending in the application.

4a) Of the above claim(s) *1-4,17 and 21* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *5, 9, 11, 13, 15-16, 18, 20, 25, 26* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Response to Amendment

1. The amendment filed 9/01/05 has been made of record. The text of those sections of Title 35, U.S. Code, not included in this action can be found in a prior office action.

Objections/Rejections: Withdrawn

Claim Rejections - 35 USC § 103

2. Rejection of claims 5, 9, 11, 18, and 25 under 35 USC § 103(a) is withdrawn due to applicant's amendment of priority.

3. Rejection of claims 13, 15, 16, 20, and 26 under 35 USC § 103(a) is withdrawn due to applicant's amendment of priority.

Objections/Rejections: Maintained/New Grounds

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. Claims 5, 9, 11,13, and 15 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably

convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

5. In particular, parts b and c of claims 5 and 13 recite both broad hybridization conditions and percentages of identity with RANK. Also, claims 11 and 15 recite polypeptides of 80% identity with that of RANK. No written description is provided in the instant specification as to what structurally constitutes polypeptide and polynucleotide sequences encompassed by hybridization conditions for polynucleotides or percent identities for the polypeptides, the boundaries and functional elements of which would be unknown to one skilled in the art at the time the invention was made. The specification has not described, nor can it be reasonably visualized by one skilled in the art the structural and functional elements attributable to a nucleic acid that hybridizes under the conditions recited. Assuming about 6X SSC and neutral pH this amounts to hybridization with sequences with as little as 63-65% (assuming approximately 1 degree centigrade lost per 1 degree of mismatch; see appendix A, page 3) identity with that of RANK, which encompasses sequences undescribed in the specification unknown in the art. Also, 80% identity of polypeptides encompasses unknown and undescribed analogs, homologs, and splice variants of RANK polypeptide with functions further undescribed in the specification or the art.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly states "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." (See page 1117.) The

specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." (See *Vas-Cath* at page 1116). As discussed above, the skilled artisan cannot envision the detailed chemical structure of the encompassed genus of polypeptides, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. The compound itself is required. See *Fiers* v. *Revel*, 25 USPQ2d 1601 at 1606 (CMC 1993) and *Amgen Inc.* v. *Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 5, 9, 11, 13, 15, 16, 18, 20, 25, and 26 rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, it is not clear what the hybridization conditions are in claims 5 and 13. For instance, what is the pH? Formamide concentration? Denhardt's concentration? Magnesium concentration? Salmon sperm DNA? Without a clear recitation of hybridization conditions the degree of hybridization with mismatched nucleic acids is indefinite.

7. Claim 5, 9, 11, 13, 15, 16, 18, 20, 25, and 26 and are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential elements, such omission amounting to a gap between the elements. See MPEP § 2172.01. The omitted elements are: washing conditions that follow hybridization conditions. Claims 5

and 13 (b) describe hybridization conditions, however omit wash conditions which are final determinants in the degree of hybridization with any nucleic acid. Claims 9, 11, 15, 16, 18, 20, 25, and 26 are rejected for depending upon an indefinite claim.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Steven Standley whose telephone number is **(571) 272-3432**. The examiner can normally be reached on Monday through Friday, 8:00 AM to 5:00 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Janet Andres can be reached on **(571) 272-0867**.

The fax number for the organization where this application or proceeding is assigned is **703-872-9306**.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see **http://pair-direct.uspto.gov**. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at **866-217-9197** (toll-free).

Steve Standley, Ph.D.
10/31/05

JANET L. ANDRES
SUPERVISORY PATENT EXAMINER

Appendix A

Molecular Biology Techniques Manual

Third Edition

Edited by:

Vernon E Coyne, M Diane James, Sharon J Reid and Edward P Rybicki

DETECTION OF NUCLEIC ACIDS BY HYBRIDISATION

Contents

INTRODUCTION

Hybridisation is a term used to describe the specific complementary association due to hydrogen bonding, under experimental conditions, of single-stranded nucleic acids. It should more properly be referred to as "annealing", as this is the physical process responsible for the association: two complementary sequences will form hydrogen bonds between their complementary bases (G to C, and A to T or U) and form a stable double-stranded, anti-parallel "hybrid" helical molecule. One may make ones nucleic acid single-stranded for the purpose of annealing - if it is not single-stranded already, like most RNA viruses - by heating it in 0.01M NaCl to a point above the "melting temperature" of the double- or partially-double-stranded form, and then flash-cooling to ±0oC: this ensures the "denatured" or separated strands do not re-anneal.

Alternatively, one may denature DNA reversibly by treatment with 0.5M NaOH: this does not work for

RNA, as this hydrolyses under these conditions.

Why would one want to anneal pieces of nucleic acid?

The answer is simple: nucleic acid hybridisation on membrane filters is a simple, sensitive, and specific means of detecting nucleic acid sequences of interest. One immobilises "target" nucleic acid - denatured so as to be effectively single-stranded - on an absorptive, porous membrane, and then anneals to it an appropriately "tagged" or "labelled" single-stranded probe nucleic acid. After washing off unannealed probe, one detects the immobilised hybrid by means of the label: this is often 32P incorporated into a nucleotide, which allows autoradiographic or scintillometric detection.

One may also use non-radioactive labels and detection systems, for sensitivities of detection down to picogram levels. The system of choice at the moment appears to be the Boehringer Mannheim DIG (digoxigenin) non-radioactive labelling and detection kit, which uses digoxigenin-11-dUTP as a substituted nucleotide which is enzymatically incorporated into DNA.

The mechanism of immobilisation of nucleic acids on membranes is not fully understood: nitrocellulose strongly binds only ss-nucleic acids (ssNA), under conditions of high salt (>1M NaCl), and has to be heated at 80oC in a vacuum to irreversibly attach the NA; nylon membranes (Hybond-N, GeneScreen) bind all nucleic acids under a wide range of salt concentrations, and irreversible or covalent attachment can be achieved by UV irradiation for 5 min or less, or by treatment with 0.4M NaOH.

The complementary association of two strands of polynucleotides

is the basis for replication of all organisms; the complexity inherent in the sequence of the molecules renders the association extremely specific for any molecule longer than sixteen nucleotides. This is easily understood if one considers the combinatorial possibilities of given lengths of "probe" sequence: there is a ¼ chance (4-1) of finding an A, G, C or T (U for RNA) in any given DNA sequence; there is a 1/16 chance (4-2) of finding any dinucleotide sequence (eg. AG); a 1/256 chance of finding a given 4-base sequence. Thus, a sixteen base sequence will statistically be present only once in every

416 bases (=4 294 967 296, or 4 billion):

this is about the size of the human genome, and 1000x greater than the genome size of *E. coli*.

Thus, the association of two nucleic acid molecules - presumed to be at least a few hundred bases long - is an extremely sequence-specific process, far more so than the widely-used specificity of monoclonal antibodies in binding to specific antigenic determinants. The correct annealing of two sequences to each other does, however, depend on the physical and chemical solution conditions under which the reaction takes place.

Melting Temperatures

For example, all double-stranded nucleic acids - whether dsDNA, dsRNA or RNA:DNA hybrids - have specific "melting temperatures", which depend mainly upon their specific guanine+cytosine content, but also upon whether they are DNA, RNA, or a mixture (RNA:RNA hybrids have the highest melting temperatures, followed by DNA:RNA hybrids, then dsDNA), and upon the ionic strength of solution.

The melting temperature is also dependent upon the length of the sequences to be annealed: the shorter the probe sequence, the lower the melting temperature. The degree of sequence mismatch also determines the effective melting temperature of a hybrid: Tm decreases by about 1oC for every 1% of mismatched base pairs. It therefore makes sense to maximise probe length in order to minimise Tm reduction due both to length and degree of sequence mismatch. Under standard conditions of annealing (0.8M NaCl, neutral pH) one may calculate the **melting temperature** ™ of any given DNA hybrid as shown:

Tm = 81.5oC + 0.41(%G + %C) - 550/n

where n=probe length (no. nucleotides).

One can see that the reduction in Tm becomes negligible for probes of length 200 nt or greater. Thus, one may vary the specificity of association of a specific single-stranded "probe" and a target by varying the incubation temperature of the annealing reaction: the higher the temperature, the higher the specificity of the reaction - and the lower the likelihood of annealing taking place.

Hybridisation Stringency

The successful use of nucleic acids as probes for sequences of interest therefore depends upon certain reaction conditions which are in turn determined by the physical properties (ie. length and sequence) of the probe. This leads to the concept of ***stringency*** of hybridisation: one increases the stringency by lessening the likelihood of non-homologous annealing. This can be done by simply increasing the temperature of incubation - bearing in mind that *rate* of hybridisation/annealing is maximal at about Tm - 25oC, and too high a temperature results in very slow annealing. An acceptable compromise is to anneal at a standard temperature (eg. 65oC), and then *wash* the annealed and immobilised hybrid molecules to varying degrees of stringency: the extent to which one should wash can be assessed by repeated autoradiography, if the probe is 32P-labelled, or by repeated colour assay of replicates in the case of non-radioactively labelled probe. Washing stringency may be increased by varying the ionic strength (from 1.0M NaCl to 0.02M), or varying the temperature (ambient to 65oC). One may also include SDS or other detergent in wash and in hybridisation buffers in order to decrease non-specific attachment of probe to the adsorptive membrane. For this reason a *blocking or prehybridisation buffer* is normally used before and during the annealing reaction, to block adsorptive sites on the membrane not occupied by target nucleic acid. This normally consists of buffer salts, detergent, protein, inert polymer material, and DNA.

It is possible to include various other constituents in annealing buffers, designed to increase the hybridisation rate, or the stringency, or both. *Formamide* is a helix destabiliser, and enables one to decrease annealing temperature: the presence of **formamide** decreases the Tm as shown:

TFm = Tm - 0.61(%formamide, w/v)

It is most often used in annealing reactions using RNA as target or probe, and especially with dsRNA hybrids, as these have high Tms which necessitate elevated reaction temperatures. Standard conditions using formamide would be 42oC with 50% formamide content in the annealing buffer. Formamide also decreases the rate of annealing, so one normally includes substances like dextran sulphate - a polyanionic polymer - as "molecular exclusion agents" to decrease the volume of solvent available to the probe. Polyethylene glycol is a far cheaper and equally effective substitute for increasing reaction rate. Too high a concentration of DS or PEG raises "background" or non-specific probe attachment to unacceptably high levels. Their effectiveness is also directly proportional to probe length, and they are useless when oligonucleotides of less than 50 nt in length are used as probes.

Summary

A standard hybridisation reaction, then, consists of ***probing*** an immobilised ***target sequence*** on a membrane with a ***labelled specific probe sequence***: this is done by annealing the probe to the target under (usually) standard "hybridisation conditions" of 0.9M NaCl, 65oC, for 4-16 hr. Probes are usually molecules of DNA or cDNA, a few hundred nt to several kilobases long, cloned into and grown up as recombinant plasmids in *E. coli*, and purified by caesium chloride gradient centrifugation. One may also use nucleic acid directly purified from the organism of interest, but this is only really effective if this is a virus or a plasmid, as otherwise the probe length is too great, and the repeat number is too small to give appreciable signal. In other words, probes should not be too long, as otherwise one needs very high concentrations of nucleic acid in order to guarantee a sufficient number of copies of the sequence in order to give a detectable "signal" for detection purposes.

Return to Molecular Biology Methods Manual
